

RAPID SCREENING METHOD OF TRANSLATIONAL FUSION PARTNERS FOR
PRODUCING RECOMBINANT PROTEINS AND TRANSLATIONAL FUSION
PARTNERS SCREENED THEREFROM

Technical Field

5 The present invention relates to a technique for
rapid screening of suitable translational fusion partners
(TFPs) capable of inducing expression or secretory
production of non-producible proteins, which are difficult
to produce using conventional recombinant production
10 methods, from a variety of genetic sources.

Background Art

There is a need to develop high-efficiency protein
production systems using recombinant microorganisms to
analyze human genome sequence data recently obtained
15 through the Human Genome Project and functions of diverse
proteins identified at genome units and to produce protein
products important in human medical fields. When an
expression system is selected to produce a recombinant
protein derived from higher organisms such as humans, a
20 variety of factors should be carefully considered, which
include growth characteristics of host cells, protein
expression levels, possibility of intracellular and

extracellular expression, possibility of posttranslational modification, biological activity of expressed proteins. As representative microbial expression systems, *E. coli* and yeast systems are mainly used. *E. coli* is advantageous because many *E. coli*-based expression systems have been developed and *E. coli* expresses heterologous proteins in high levels. However, *E. coli* has the following drawbacks: the inability to perform posttranslational modification for recombinant production of proteins derived from higher eukaryotes, the difficulty in complete secretion of proteins into the culture medium, the lack of folding ability of proteins possessing many disulfide bonds, and the expression of proteins in insoluble forms such as inclusion bodies (Makrides, Microbial Rev., 1996, 60, 512). In addition, since medically valuable disease-associated proteins among human proteins are mostly glycoproteins or membrane proteins, they need glycosylation and folding into a correct three-dimensional structure through disulfide bonds in order to achieve full activity. Thus, these proteins are impossible to produce in *E. coli* and essentially require eukaryotic expression systems such as yeasts.

Yeast *Saccharomyces cerevisiae* is a eukaryotic microorganism proven to be safe to the human body as a GRAS (Generally Recognized As Safe) organism. *S. cerevisiae* has many advantages including easy gene manipulation, various developed expression systems and easy large-scale culture.

The advantages further include that *S. cerevisiae* functions to secrete higher cell-derived proteins such as human proteins into the extracellular space, and performs posttranslational modification of proteins, such as glycosylation. The extracellular secretion can be achieved through the artificial fusion of a target protein with a protein secretory signal, and during the secretion of a protein, protein folding or disulfide bond formation and glycosylation occur, thereby producing a fully biologically active recombinant protein. Also, since a biologically active protein can be obtained directly from the culture medium, *S. cerevisiae*-based protein expression systems do not require cost-inefficient cell disruption or refolding so that they are very economical (Eckart and Bussineau, Curr. Opin. Biotechnol., 1996, 7, 525).

However, despite the many advantages of *S. cerevisiae* mentioned above, the problem of present techniques associated with systems for secreting human proteins using yeast *S. cerevisiae* involves non-uniform protein secretion yield ranging from no production to several grams/liter, depending on the human protein, leading to a great difference of more than several thousands in protein secretion yield, thus making it difficult to predict secretion yield. When a heterologous protein is secreted in several grams/liter, this protein production is considered to be cost-effective. In contrast, for the production of proteins expressed in low

levels, especially highly valuable human therapeutic proteins, difficulties often occur in the expression and secretion of the proteins. To solve these problems, much research has been focused on secretory factors involved in protein secretion. For example, many studies have been carried out on chaperons, including a method of overexpressing a secretory factor, BiP (*KAR2*), which helps fold proteins newly synthesized in the endoplasmic reticulum(ER) (Robinson et al., *Biotechnol. prog.*, 1996, 271, 10017), and a method of overexpressing *PDI* (protein disulfide isomerase) helping the formation of cysteine bonds (Robinson et al., *Bio/Technology*, 1994, 12, 381; Schultz et al., *Ann. N. Y. Acad. Sci.*, 1994, 721, 148; Hayano et al., *FEBS Lett.*, 1995, 377, 505). Also, another study has been performed to improve secretion through preparation of a fusion partner inducing secretion and fusion with a well-secreted protein (Gouka et al., *Appl. Microbiol. Biotechnol.*, 1997, 47, 1). To date, these methods have been considered to be very successful in improving the secretion of heterologous proteins. Molecular mechanisms of these fusion techniques have been poorly studied, but these fusion techniques have been experimentally proven to reduce limitations in translational or posttranslational steps, including facilitating protein translocation and helping protein folding.

Kjeldsen et al. (*Protein Expr. Purif.*, 1997, 9, 331)

enhanced the secretion of insulin by fusing insulin precursor with a synthetic leader prepared based on theoretical consideration in order to achieve effective secretion of insulin or insulin precursor. The synthetic leader has an N-glycosylation site and a BiP recognition site so that it extends the residence of the fusion protein in the ER, leading to correct folding of the insulin precursor. Also, the synthetic leader in which an additional glycosylation site is introduced remarkably increased the secretion of insulin in *Aspergillus niger* and *Saccharomyces cerevisiae* (Kjeldsen et al., *Protein Expr. Purif.*, 1998, 14, 309). Similar results were obtained in *Aspergillus awamori* (Ward et al., *Bio/Technology*, 1989, 8, 435) and when hydrophobic cutinase is expressed in yeast (Sagt et al., *Appl. Environ. Microbiol.* 2000, 66, 4940). This high-yield secretion of recombinant proteins results from the introduction of glycosylation sites that increase the solubility of recombinant proteins in the ER and induce correct folding of the proteins.

Well-secreted proteins have been employed as fusion partners. For example, fusion expression with glucoamylase from *Aspergillus awamori* resulted in an increase in secretion yield of the following proteins: bovine prochymosin (Ward et al., *Bio/Technology*, 1989, 8, 435), porcine pancreatic phospholipase A2 (Roberts et al., *Gene*, 1992, 122, 155), human interleukin-6 (Contreras et al., *Bio/Technology* 1991,

9, 378; Broekhuijsen et al., J. Biotechnol., 1993, 31, 135), hen egg-white lysozyme (Jeenes et al., FEMS Microbiol Lett, 1993, 107, 267), and human lactoferrin (Ward et al., Bio/Technology, 1995, 13, 498). Increased secretion yield 5 varied, depending on the protein, in a range of 5 to 1000 times. Also, the use of amino-terminal 24 amino acids of human interleukin-1 β as a fusion partner in yeast resulted in an increase in secretion yield of human growth hormone and granulocyte colony-stimulating factor (G-CSF) (Lee et al., 10 Biotechnol. Prog., 1999, 15, 884). Human interleukin-1 β is secreted without a particular secretory signal (Muesch et al., Trends Biochem. Sci., 1990, 15, 86), and its recombination production is very effective via secretion in yeast (Baldari et al., Protein Eng., 1987, 1, 433). Also, 15 according to a recent report, a fusion partner originally retained in a protein is essential for correct folding of the protein (Takahashi et al., Appl Microbiol. Biotechnol., 2001, 55, 454). When the mature form of *Rhizopus oryzae* lipase (ROL) fused to the pre-pro-leader sequence of the mating 20 factor alpha from *S. cerevisiae* was expressed in order to express ROL in *S. cerevisiae*, secretion of ROL was not observed. However, when ROL was synthesized together with the prosequence, ROL was properly secreted. These results demonstrate that the prosequence of ROL is essential for the 25 folding of ROL itself.

As described above, through much research, various

secretory factors have been developed to induce the secretion of recombinant proteins. However, although the developed secretory factors are effective to increase the secretion level of particular proteins, they cannot be used as a general means for the secretory production of all proteins.

Dorner et al. reported that overexpression of BiP in CHO cells rather reduces protein secretion (Dorner et al., EMBO J., 1992, 11, 1563), and decreased BiP expression increases protein secretion (Dorner et al., Mol. cell. Biol., 1988, 8, 4063). In yeast, overexpression of KAR2 (BiP) did not enhance the secretion of plant thaumatin (Harmsen et al., Appl. Microbiol. Biotechnol., 1996, 46, 365). Overexpression of BiP in Baculovirus resulted in an increase in levels of a soluble antibody in cell lysates but did not increase secretion yield of the antibody (Hsu et al., Protein Expr. Purif., 1994, 5, 595). When another secretory factor PDI as a foldase was overexpressed in *Aspergillus niger*, secretion of glucoamylase did not increase (Wang and Ward, Curr. Genet. 2000, 37, 57). Secretion improvement using a protein fusion partner was also reported to have a problem of increasing the secretion efficiency only of particular proteins.

Disclosure of the Invention

As described above, much research has been focused on the effects of secretory factors, but secretory factors

have different effects on secretion level depending on the types of proteins and thus cannot be applied to all proteins. Thus, there is a need for a technique of screening an optimal secretory factor specifically applicable to a target protein for maximal secretion of the target protein. In this regard, the present inventors developed a technique of rapidly screening an optimal secretory fusion partner from a genome unit according to types of recombinant proteins.

Accordingly, the present invention aims to provide a method capable of rapidly screening a suitable translational fusion partner (TFP) capable of strongly inducing production of a protein, which is unable to be produced at large scale and low cost due to its low expression levels in yeasts, from a variety of genetic sources including yeasts, and a translational fusion partner capable of stimulating the secretory production of a non-producible protein using the method.

Brief Description of the Drawings

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

FIG. 1 shows a process of deleting the invertase gene

and a pop-out process of a selectable marker;

FIG. 2 shows zymogram analysis for invertase activity (lanes 1, 2 and 3: wild-type *Saccharomyces cerevisiae* Y2805; and lanes 4, 5 and 6: invertase-deficient strain (*S. cerevisiae* Y2805 Δ inv2);

FIG. 3 photographically shows the growth of yeast cells according to carbon sources (INV2: wild-type *S. cerevisiae* Y2805; and Δ inv2: invertase-deficient strain (*S. cerevisiae* Y2805 Δ inv2)

FIG. 4 shows the results of Southern blotting for the deletion of the invertase gene (lanes 1 and 2: *S. cerevisiae* Y2805 ura3 INV2; lanes 3 and 4: *S. cerevisiae* Y2805 Δ inv2U (URA3 Δ inv2); and lanes 5 and 6: *S. cerevisiae* Y2805 Δ inv2 (ura3 Δ inv2);

FIG. 5 photographically shows the growth of yeast cells on glucose and sucrose media;

FIG. 6 shows a process of preparing pYHTS-F0, F1 and F2 plasmids and a process of preparing a yeast gene library;

FIG. 7 shows the results of SDS-PAGE and Western blotting for culture supernatants of yeast cells containing any of four translational fusion partners (lane 1: size marker; lane 2: interleukin-2; lane 3: culture supernatant of yeast cells containing pYIL-TFP1; lane 4: culture supernatant of yeast cells containing pYIL-TFP2; lane 5: culture supernatant of yeast cells containing pYIL-TFP3; and lane 6: culture supernatant of yeast cells containing pYIL-TFP4);

FIG. 8 shows the results of glycosylation analysis by Endo-H digestion, wherein samples are analyzed on SDS-PAGE (lane 1 (-): culture supernatant of yeast cells containing pYIL-TFP1, not treated with Endo-H; lane 1 (+): culture supernatant of yeast cells containing pYIL-TFP1, treated with Endo-H; lane 2 (-): culture supernatant of yeast cells containing pYIL-TFP3, not treated with Endo-H; lane 2 (+): culture supernatant of yeast cells containing pYIL-TFP3, treated with Endo-H; lane 3 (-): culture supernatant of yeast cells containing pYIL-TFP4, not treated with Endo-H; and lane 3 (+): culture supernatant of yeast cells containing pYIL-TFP4, treated with Endo-H);

FIG. 9 shows the results of SDS-PAGE of culture supernatants of yeast cells according to the presence or absence of a Kex2p possessing site (lane M: size marker, lane 1: culture supernatant of yeast cells containing pYIL-TFP1; lane 2: culture supernatant of yeast cells containing pYIL-KRTFP1; lane 3: culture supernatant of yeast cells containing pYIL-TFP3; lane 4: culture supernatant of yeast cells containing pYIL-KRTFP3; lane 5: culture supernatant of yeast cells containing pYIL-TFP4; and lane 6: culture supernatant of yeast cells containing pYIL-KRTFP4);

FIG. 10 is a schematic presentation of plasmids from which the TFP1 gene has been partially deleted for the analysis of characteristics of TFP1;

FIG. 11 shows the results of SDS-PAGE for analyzing

the ability of TFP1-derived translational fusion partners (TFP-1, 2, 3 and 4) to secrete interleukin-2 (lane M: size marker; lane S: interleukin-2; lane 1-1: culture supernatant of yeast cells containing pYIL-KRT1-1(also, referred to as 5 pYIL-KRTFP1-1); lane 1-2: culture supernatant of yeast cells containing pYIL-KRT1-2(also, referred to as pYIL-KRTFP1-2); lane 1-3: culture supernatant of yeast cells containing pYIL-KRT1-3(also, referred to as pYIL-KRTFP1-3); lane 1: culture supernatant of yeast cells containing pYIL-KRTFP1; and lane 10 1-4: culture supernatant of yeast cells containing pYIL-KRT1-4(also, referred to as pYIL-KRTFP1-4));

FIG. 12 shows a profile for fed-batch fermentation of a recombinant yeast strain containing pYIL-KRT1-4 and the results of SDS-PAGE for analyzing proteins secreted into the 15 medium according to fermentation time;

FIG. 13 shows the results of SDS-PAGE and Western blotting, displaying that translational fusion partners TFP1, 2, 3 and 4 induce the secretion of a non-producible protein human G-CSF (lane M: size marker; lane 1: culture supernatant 20 of yeast cells containing pYGCSF-KRTFP1; lane 2: culture supernatant of yeast cells containing pYGCSF-KRTFP2; lane 3: culture supernatant of yeast cells containing pYGCSF-KRTFP3; and lane 4: culture supernatant of yeast cells containing pYGCSF-KRTFP4);

FIG. 14 shows a profile for fed-batch fermentation of 25 a recombinant yeast strain containing pYGCSF-TFP3 and the

results of SDS-PAGE for analyzing proteins secreted into the medium according to fermentation time;

FIG. 15 shows the isolation of the full-length gene of which a translational fusion partner TFP3 is a part and the enhanced secretion of G-CSF through the modification of a fusion site between the isolated gene and the G-CSF gene ((A) lane T3: culture supernatant of yeast cells containing pYGCSF-KRTFP3, lane T3-1: culture supernatant of yeast cells containing pYGCSF-KRTFP3-1, lane T3-2: culture supernatant of yeast cells containing pYGCSF-KRTFP3-2, lane T3-3: culture supernatant of yeast cells containing pYGCSF-KRTFP3-3, and lane T3-4: culture supernatant of yeast cells containing pYGCSF-KRTFP3-4; and (B) lane T3: culture supernatant of yeast cells containing pYGCSF-KRTFP3, lane T3-1: culture supernatant of yeast cells containing pYGCSF-KRTFP3-1, lane T3-1-1: culture supernatant of yeast cells containing pYGCSF-KRTFP3-1-1, lane T3-1-2: culture supernatant of yeast cells containing pYGCSF-KRTFP3-1-2, and lane T3-2: culture supernatant of yeast cells containing pYGCSF-KRTFP3-2);

FIG. 16 shows the results of SDS-PAGE of fermentation media for the secretory production of an industrial enzyme CalB14 by a translational fusion partner TFP3 (lane M: size marker; lanes 1 and 2: culture supernatant of yeast cells containing pYGA-CalB14 at a low temperature of 20°C; and lanes 12-58: culture supernatant of yeast cells containing pYGT3-CalB14);

FIG. 17 shows the results of SDS-PAGE of samples collected at given time points upon fetch culture of *Pichia pastoris* containing a G-CSF expression vector containing a translational fusion partner TFP1, pGAP-TFP1-GCSF, or a conventional G-CSF expression vector pGAP-MFalpha-GCSF (lane Sc: 10 µl of a culture supernatant of yeast *S. cerevisiae* containing pYCSF-KRTFP1; and lanes 6-24: concentrates of 200 µl of culture supernatants of *P. pastoris* containing pGAP-TFP1-GCSF or pGAP-MFalpha-GCSF);

FIG. 18 is a schematic map of pYIL-KRTFP1;
FIG. 19 is a schematic map of pYIL-KRTFP2;
FIG. 20 is a schematic map of pYIL-KRTFP3;
FIG. 21 is a schematic map of pYIL-KRTFP4;
FIG. 22 is a schematic map of pYIL-KRT1-3(also,
referred to as pYIL-KRTFP1-3);
FIG. 23 is a schematic map of pYIL-KRT1-4(also,
referred to as pYIL-KRTFP1-4);
FIG. 24 is a schematic map of pYGT3-1-1-GCSF; and
FIG. 25 is a schematic map of pYGT3-1-2-GCSF.

20 Best Mode for Carrying Out the Invention

In one aspect, the present invention relates to a method of screening, from a gene library, a translational fusion partner (TFP) that induces extracellular secretion of a non-producible X-R fusion protein, which is prepared

by linking a non-producible target protein gene (X) to a reporter gene (R) for automatic screening, through fusion of the X-R fusion product with other genes.

In one detailed aspect, the present invention relates 5 to a method of screening a suitable translational fusion partner (TFP) for producing a non-producible protein, comprising:

(1) preparing an automatic screening vector including 10 a fusion gene (X-R) in which a gene (X) encoding a non-producible target protein is linked in frame to a reporter gene (R) for automatic screening;

(2) linking a gene library including a TFP inducing secretion of the non-producible fusion protein (X-R) to the automatic screening vector to create a TFP library;

15 (3) transforming cells having no activity of the reporter gene with the TFP library to detect the activity of a reporter protein; and

(4) isolating a gene from transformed cells exerting 20 the activity of the reporter protein and analyzing properties of the TFP.

The term "translational fusion partner (TFP)", as used herein, refers to a gene that is fused to a gene encoding a non-producible protein and induces the secretory production of the non-producible protein. Also, the 25 "translational fusion partner protein" means a protein having an amino acid sequence encoded by the aforementioned

TFP gene.

The term "non-producible protein", as used herein, refers to a protein that is difficult to express in host cells, such as *E. coli* or yeasts, due to its native properties with respect to recombinant production of proteins from humans or various organisms. In particular, with respect to the objects of the present invention, a non-producible protein is a protein that is difficult to express in eukaryotic host cells such as yeasts in recombinant production. The screening method of the present invention and the translational fusion partner obtained using the screening method are used for recombinant production of proteins that cannot be recombinantly produced in both prokaryotic cells such as *E. coli* and eukaryotic cells such as yeasts, as well as a plurality of proteins that can be recombinantly produced in prokaryotic cells such as *E. coli* but are cost-ineffective due to their low yield in eukaryotic cells such as yeasts. As used herein, the term "expression" means that a transcriptional and translational product of a gene encoding a particular protein is secreted and obtained as a final desired product.

The reporter gene for automatic screening according to the present invention is selected from, but is not limited to, a gene group encoding proteins capable of being extracellularly secreted, including invertase, sucrase,

cellulase, xylanase, maltase, amylase, glucoamylase and galactosidase.

In the screening method, the gene library including a translational fusion partner for inducing the secretion of 5 a non-producible fusion protein may be obtained from a variety of origins, for example, animals, plants and microorganisms, including yeasts or humans. Preferred is a gene library from yeasts. The yeasts for the gene library includes *Candida*, *Debaryomyces*, *Hansenula*, *Kluyveromyces*, 10 *Pichia*, *Schizosaccharomyces*, *Yarrowia*, *Saccharomyces*, *Aspergillus*, *Penicillium*, *Rhizopus*, *Trichoderma*, etc. The gene library may be in the form of genomic (chromosomal) DNA or cDNA.

In one embodiment, when a non-producible protein (X), 15 which is difficult to express in recombinant production, is fused to an invertase (I) and expressed in yeast cells, because the secretion of invertase secreted under normal conditions is inhibited by the fused poorly-secreted protein (X), the yeast cells do not grow or their growth is 20 greatly delayed, due to poor expression levels of the fusion protein on a medium containing only sucrose as a carbon source. However, when an effective translational fusion partner capable of inducing the expression and secretion of X-I is introduced, cells rapidly grow on a 25 sucrose medium. Based on this principle, when the X-I fusion protein of a non-producible protein and an invertase

is additionally fused to a translational fusion partner library obtained from a variety of origins in the form of TFP-X-I or X-I-TFP, introduced into yeast cells and expressed therein, cells rapidly growing on the sucrose medium are selected, thereby allowing rapid screening of a TFP most suitable for the non-producible protein from a variety of libraries.

Therefore, in a more preferred aspect, the present invention relates to a method of rapidly screening a suitable translational fusion partner (TFP) for producing a non-producible protein, comprising:

(1) preparing a yeast mutant strain deleted for its endogenous invertase gene INV2(I) to develop an automatic screening system using a yeast invertase as a reporter gene;

(2) preparing yeast high-throughput selection (HTS) vectors, pYHTS vectors (pYHTS-F0, pYHTS-F1 or pYHTS-F2) containing a gene (X-I) in which an invertase gene (I) is fused in frame to a non-producible protein gene (X) and which is controlled in expression under a yeast GAL10 promoter;

(3) preparing a translational fusion partner library from yeast genes capable of secreting the fusion gene (X-I) of an invertase and a non-producible protein in the pYHTS vectors;

(4) transforming the library into the yeast mutant

strain prepared at step (1) and performing automatic screening on a medium containing only sucrose as a carbon source;

(5) detecting a protein secreted into the medium by
5 culturing yeast cells grown on the sucrose medium; and

(6) isolating genes from the yeast cells and analyzing properties of the translational fusion partner.

The present inventors prepared an invertase-deficient yeast mutant and found that invertase can be used as a
10 marker for automatic screening through the expression of a protein fused to the invertase in a yeast strain deleted for its invertase gene. Then, the present inventors prepared vectors for the automatic screening of a translational fusion partner, pYHTS-F0, F1 and F2, using a
15 non-producible protein, human interleukin-2, linking yeast-derived cleaved chromosomal DNA to the vectors to generate a translational fusion partner library, and found, from the TFP library, TFP proteins suitable for the poorly secreted protein human interleukin-2, TFP1, TFP2, TFP3 and TFP4.

20 Yeast cells need an invertase enzyme encoded by a yeast *INV2* gene using only sucrose as a carbon source. As used herein, the term "automatic screening system using invertase" means a system for selecting a yeast strain growing on a sucrose medium according to the expression of
25 an *INV2* gene introduced into a vector while the yeast strain is deleted for its chromosomal *INV2* gene.

The enzyme invertase has been used as a reporter protein. For example, U.S. Pat. No. 6,228,590 and EP 0 907 727 B1 disclose methods of screening fusion partners inducing the secretion of invertase lacking the native secretory signal sequence and thus not being secreted. In contrast, the present invention employs the invertase enzyme for screening a translational fusion partner capable of inducing the expression of a non-producible fusion protein in which the invertase enzyme is fused to a non-
5 producible protein. As a result, the number of transformants expressing invertase was remarkably reduced, thereby giving better distinguish discrimination between true and false positives. Thus, the present invention allows rapid identification of a translational fusion
10 partner specifically applicable to a non-producible protein.
15

Translational fusion partners TFP1, TFP2, TFP3 and TFP4 and derivatives thereof, which are obtained in the present invention, may be applied to a variety of proteins produced at commercial large scale. These proteins include,
20 but are not limited to, cytokines (e.g., interleukin), serum proteins (e.g., coagulation factors including Factors VII, VIII and IX), immunoglobulins, cytokine receptors, lactoferrin, interferons (e.g., interferon- α , - β and - γ), colony stimulating factors (e.g., GM-CSF, G-CSF),
25 phospholipase activating protein (PLAP), insulin, tumor

necrosis factor (TNF), growth factors (e.g., tissue growth factors and epithelial growth factors, such as TGF α or TGF β , epidermal growth factor (EGF), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF)),
5 hormones (e.g., follicle stimulating hormone, thyroid stimulating hormone, antidiuretic hormone, pigmentary hormone and parathyroid hormone, luteinizing hormone-releasing hormone and derivatives thereof), calcitonin, calcitonin gene related peptide (CGRP), enkephalin,
10 somatomedin, erythropoietin, hypothalamic releasing factor, prolactin, chorionic gonadotropin, tissue plasminogen activator, growth hormone releasing peptide (GHRP), thymic humoral factor (THF), and anticancer and antibiotic peptides. Also, these proteins may include enzymes, which
15 are exemplified by carbohydrate-specific enzymes, proteolytic enzymes, lipases, oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Concrete examples of enzymes include, but are not limited to, asparaginase, arginase, arginine deaminase, adenosine
20 deaminase, peroxide dismutase, endotoxinase, catalase, chymotrypsin, uricase, adenosine diphosphatase, tyrosinase, and bilirubin oxidase. Examples of the carbohydrate-specific enzymes include glucose oxidase, glucodase, galactosidase, glucocerebrosidase and glucuronidase.

25 Non-producible protein genes are genes that encode the aforementioned proteins having human medical or

industrial importance, the recombinant production of which is required, and are isolated or chemically synthesized from genes derived from a variety of plants, animals and microorganisms, including humans.

5 The automatic screening vector of the present invention includes a promoter gene, a gene encoding a target protein, which is deleted for translation initiation and termination codons, and a reporter gene fused in frame to the gene encoding the target protein. The promoter gene
10 is preferably selected from the group consisting of *GAPDH*, *PGK*, *ADH*, *PHO5*, *GAL1* and *GAL10*.

In the automatic screening method of the present invention, the host cells to be transformed include, but are not limited to, yeasts, such as *Candida*, *Debaryomyces*,
15 *Hansenula*, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces*, *Yarrowia* and *Saccharomyces* species, fungi, such as *Aspergillus*, *Penicillium*, *Rhizopus* and *Trichoderma* species, and bacteria, such as *Escherichia* and *Bacillus* species.

The rapid screening method of a suitable TFP for
20 producing a non-producible protein according to the present invention is preferably used for producing non-producible proteins that are not expressed or are expressed in low levels. Also, the present method may be used for screening a TFP capable of increasing expression levels of a low-
25 level expressed protein. As in one embodiment of the present invention, when the invertase enzyme is used as a

reporter, cells are selected according to their growth rates on the sucrose medium, thereby allowing discrimination of more effective TFPs.

In another aspect, the present invention relates to vectors, pYHTS-F0, F1 and F2, for rapidly screening a suitable fusion partner for stimulating the secretory production of a non-producible protein interleukin-2. These screening vectors include a fusion gene of the non-producible protein human interleukin-2 and invertase, and contain a *BamHI* recognition site having three different reading frames at an amino terminal end of the interleukin-2 gene.

In an embodiment of the present invention, in order to rapidly screen suitable fusion partners stimulating the secretory production of human interleukin-2 in yeast cells, yeast chromosomal DNA is randomly cleaved and inserted into the three screening vectors (pYHTS-F0, F1 and F2). A yeast strain lacking invertase is transformed with the resulting screening vectors, and colonies growing on a sucrose medium are selected to identify suitable fusion partners capable of secreting the fusion protein of non-producible interleukin-2 and invertase into the culture medium.

Human interleukin-2, which is a highly hydrophobic protein, is difficult to express in yeast cells because the recombinant protein expressed at large scale by a strong promoter is not folded rapidly into an active form in the

ER but forms aggregates that may block the function of the ER. Thus, when fused to interleukin-2, invertase is also not secreted, and yeast cells cannot grow on a sucrose medium. Translational fusion partners capable of effectively secreting this fusion protein may be identified by inserting a yeast genomic library upstream of the interleukin-2 gene, transforming the library into a yeast strain and selecting transformants growing on a sucrose medium.

In an embodiment, in order to obtain fusion partners inducing secretion of the non-producible protein interleukin-2, the present inventors isolated genes from transformants growing on a sucrose medium, re-transformed the genes into *E. coli*, and recovered four different plasmids (pYHTS-TFP1, TFP2, TFP3 and TFP4). Four different translational fusion partner genes carried in the plasmids, TFP1 (SEQ ID NO. 2), TFP2 (SEQ ID NO. 4), TFP3 (SEQ ID NO. 6) and TFP4 (SEQ ID NO. 8), were obtained, and corresponding amino acid sequences are represented by SEQ ID NOS. 1, 3, 5 and 7, respectively.

The invertase gene was deleted in the obtained vectors pYHTS-TFP1, TFP2, TFP3 and TFP4, and a translation termination codon was inserted into the interleukin-2 gene, thus generating pYIL-TFP1, TFP2, TFP3 and TFP4. Since these vectors secrete interleukin-2 in the form of being fused to a translational fusion partner, a recognition site for

Kex2p proteinase is inserted to allow automatic removal of the translational fusion partner, thus generating pYIL-KRTFP1, KRTFP2, KRTFP3 and KRTFP4. Also, human granulocyte colony stimulating factor (G-CSF) is fused to the 5 translational fusion partners TFP1 to TFP4, thus generating vectors pYGCSF-TFP1 to pYGCSF-TFP4, respectively. These vectors demonstrated that the TFPs are effective in secretory production of proteins other than human interleukin-2.

On the other hand, when a conventional expression-secretion system (AMY, amylase secretory signal) is used for large-scale secretory production in a recombinant yeast strain, a CalB14 mutant having about 6-fold improved specific activity through molecular evolution of wild-type 15 *Candida antarctica* lipase B (CalB) that has attracted much interest due to its potential use in industrial applications, the CalB14 is not effectively secreted at an optimal yeast culture temperature of 30°C but is secreted at a low temperature of 22°C. Since the conventional system 20 has problems of low growth rates of yeast in large-scale fermentation and high cost for temperature control of a fermentor, especially in the summer, there is a need for a secretion system allowing secretory production at an optimal culture temperature. In the present invention, 25 these problems are solved by preparing a pYGT3-CalB4 vector carrying CalB fused to TFP3.

Thus, in a further aspect, the present invention relates to a translational fusion partner TFP1 protein represented by SEQ ID NO. 1 or an analogue thereof. Also, the present invention relates to a gene encoding a 5 translational fusion partner TFP1 protein represented by SEQ ID NO. 1 or an analogue thereof. The scope of the present invention includes a translational fusion partner TFP1 protein having an amino acid sequence represented by SEQ ID NO. 1 or an amino acid sequence homologous thereto, 10 having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. Also, the scope of the present invention includes a gene having a DNA sequence encoding a translational fusion partner TFP1 protein represented by SEQ ID NO. 1, or a DNA 15 sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. Preferably, the gene is a gene of SEQ ID NO. 2. Further, the present invention relates to a recombinant vector comprising the gene. 20 Preferably, the gene carried in the recombinant vector is a gene of SEQ ID NO. 2. Examples of the recombinant vector include pYIL-TFP1, pYIL-KRTFP1, pYGCSF-TFP1, pYGCSF-KRTFP1 and pGAP-TFP1-GCSF. Still further, the present invention relates to a cell transformed with the recombinant vector. 25 *Escherichia coli* transformed with pYIL-KRTFP1 was deposited at KCTC (Korean Collection for Type Cultures) on November

11, 2003, and assigned accession number KCTC 10544BP.

In still another aspect, the present invention relates to a translational fusion partner TFP2 protein represented by SEQ ID NO. 3 or an analogue thereof. Also, 5 the present invention relates to a gene encoding a translational fusion partner TFP2 protein represented by SEQ ID NO. 3 or an analogue thereof. The scope of the present invention includes a translational fusion partner TFP2 protein having an amino acid sequence represented by 10 SEQ ID NO. 3 or an amino acid sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. Also, the scope of the present invention includes a gene having a DNA sequence encoding a translational fusion 15 partner TFP2 protein represented by SEQ ID NO. 3, or a DNA sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. Preferably, the gene is a gene of SEQ ID NO. 4. Further, the present invention 20 relates to a recombinant vector comprising the gene. Preferably, the gene carried in the recombinant vector is a gene of SEQ ID NO. 4. Examples of the recombinant vector include pYIL-TFP2, pYIL-KRTFP2, pYGCSF-TFP2 and pYGCSF-KRTFP2. Still further, the present invention relates to a 25 cell transformed with the recombinant vector. *Escherichia coli* transformed with pYIL-KRTFP2 was deposited at KCTC

(Korean Collection for Type Cultures) on November 11, 2003, and assigned accession number KCTC 10545BP.

In still another aspect, the present invention relates to a translational fusion partner TFP3 protein represented by SEQ ID NO. 5 or an analogue thereof. Also, the present invention relates to a gene encoding a translational fusion partner TFP3 protein represented by SEQ ID NO. 5 or an analogue thereof. The scope of the present invention includes a translational fusion partner TFP3 protein having an amino acid sequence represented by SEQ ID NO. 5 or an amino acid sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. Also, the scope of the present invention includes a gene having a DNA sequence encoding a translational fusion partner TFP3 protein represented by SEQ ID NO. 5, or a DNA sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. Preferably, the gene is a gene of SEQ ID NO. 6. Further, the present invention relates to a recombinant vector comprising the gene. Preferably, the gene carried in the recombinant vector is a gene of SEQ ID NO. 6. Examples of the recombinant vector include pYIL-TFP3, pYIL-KRTFP3, pYGCSF-TFP3, pYGCSF-KRTFP3 and pYGT3-CalB14. Still further, the present invention relates to a cell transformed with the recombinant vector.

Escherichia coli transformed with pYIL-KRTFP3 was deposited at KCTC (Korean Collection for Type Cultures) on November 11, 2003, and assigned accession number KCTC 10546BP.

In still another aspect, the present invention
5 relates to a translational fusion partner TFP4 protein represented by SEQ ID NO. 7 or an analogue thereof. Also, the present invention relates to a gene encoding a translational fusion partner TFP4 protein represented by SEQ ID NO. 7 or an analogue thereof. The scope of the
10 present invention includes a translational fusion partner TFP4 protein having an amino acid sequence represented by SEQ ID NO. 7 or an amino acid sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology.
15 Also, the scope of the present invention includes a gene having a DNA sequence encoding a translational fusion partner TFP4 protein represented by SEQ ID NO. 7, or a DNA sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most
20 preferably 95% or higher homology. Preferably, the gene is a gene of SEQ ID NO. 8. Further, the present invention relates to a recombinant vector comprising the gene. Preferably, the gene carried in the recombinant vector is a gene of SEQ ID NO. 8. Examples of the recombinant vector
25 include pYIL-TFP4, pYIL-KRTFP4, pYGCSF-TFP4 and pYGCSF-KRTFP4. Still further, the present invention relates to a

cell transformed with the recombinant vector. *Escherichia coli* transformed with pYIL-KRTFP4 was deposited at KCTC (Korean Collection for Type Cultures) on November 11, 2003, and assigned accession number KCTC 10547BP.

5 The term "analogue", as used for a translational fusion partner protein or gene herein, means a functional equivalent that exerts the activity of the translational fusion partner by inducing secretory production of a non-producible protein when a translational fusion partner gene
10 is fused to a gene encoding the non-producible protein. In the case of the TFP protein, the analogue may include, for example, substitutions between amino acids having the same properties (e.g., replacement of a hydrophobic amino acid with another hydrophobic amino acid, replacement of a hydrophilic amino acid with another hydrophilic amino acid,
15 replacement of a basic amino acid with another basic amino acid, replacement of an acidic amino acid with another acidic amino acid), deletions and insertions of amino acids, or combinations thereof.

20 With respect to substitution analogues of the translational fusion partner proteins of the present invention, amino acid substitutions in proteins and peptides which do not generally alter the activity of the proteins or peptides are known in the art (H. Neurath, R.
25 L. Hill, *The Proteins*, Academic Press, New York, 1979). The most commonly occurring substitutions are Ala/Ser, Val/Ile,

Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Ser/Gly, Thy/Phe, Ala/Pro, Lys/Arg, Asp/Asn, Leu/Ile, Leu/Val, Ala/Glu and Asp/Gly, in both directions.

In addition, with respect to deletion analogues of
5 the translational fusion partner proteins of the present invention, the deletion of a portion of the whole sequence of the translational fusion partner genes identified using a genomic library (chromosomal library) or a cDNA library may not affect, or may stimulate, the secretion of a non-
10 producible protein. The present inventors investigated the effect of deletion analogue fragments of the translational fusion partners TFP1, TFP2, TFP3 and TFP4 on the secretion of a non-producible protein. A vector carrying TFP1 that is deleted in a serine/alanine-rich sequence, an N-
15 glycosylation site or both did not secrete a non-producible protein. In contrast, a vector (pYIL-KRT1-4) carrying TFP1 that has been deleted in the 5'-UTR (5'-untranslated region) increased expression levels of the non-producible protein over three times. Also, when the 3'- end was additionally
20 deleted (pYIL-KRT1-3), the secretion of the non-producible protein was induced. Thus, deletion analogues of the translational fusion partners of the present invention in a 5'-UTR and partially deleted TFPs in 3'-end are included in the scope of the present invention, as long as they do not
25 negatively affect the secretion of a non-producible protein.

In a detailed aspect, the present invention provides a translational fusion partner TFP1-3 protein represented by SEQ ID NO. 9 or an analogue thereof. Also, the present invention provides a gene encoding a translational fusion partner TFP1-3 protein represented by SEQ ID NO. 9 or an analogue thereof. The scope of the present invention includes a translational fusion partner TFP1-3 protein having an amino acid sequence represented by SEQ ID NO. 9 or an amino acid sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. Also, the scope of the present invention includes a gene having a DNA sequence encoding a translational fusion partner TFP1-3 protein represented by SEQ ID NO. 9, or a DNA sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. Further, the present invention provides a recombinant vector comprising the gene. Preferably, the gene carried in the recombinant vector is a gene encoding a translational fusion partner TFP1-3 represented by SEQ ID NO. 9. An illustrative example of the recombinant vector is pYIL-KRT1-3(also, referred to as pYIL-KRTFP1-3). Still further, the present invention relates to a cell transformed with the recombinant vector. *Escherichia coli* transformed with pYIL-KRT1-3 was deposited at KCTC (Korean Collection for Type Cultures) on November 11, 2003, and

assigned accession number KCTC 10548BP.

In another detailed aspect, the present invention relates to a translational fusion partner TFP1-4 protein encoded by a gene represented by SEQ ID NO. 10 or an analogue thereof. Also, the present invention relates to a gene encoding a translational fusion partner TFP1-4 and represented by SEQ ID NO. 10 or an analogue thereof. The scope of the present invention includes a translational fusion partner TFP1-4 protein having an amino acid sequence 5 encoded by a gene represented by SEQ ID NO. 10 or an amino acid sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. Also, the scope of the present invention includes a gene having a DNA sequence 10 represented by SEQ ID NO. 10, or a DNA sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. The present invention provides a recombinant vector comprising a gene encoding a translational fusion 15 partner TFP1-4 and represented by SEQ ID NO. 10 or an analogue thereof. Also, the present invention provides a recombinant vector comprising a gene having a DNA sequence encoding a translational fusion partner TFP1-4 and represented by SEQ ID NO. 10 or a DNA sequence homologous 20 thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. The present invention provides a recombinant vector comprising a gene having a DNA sequence 25 encoding a translational fusion partner TFP1-4 and represented by SEQ ID NO. 10 or a DNA sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher

homology. An illustrative example of the recombinant vector is pYIL-KRT1-4(also, referred to as pYIL-KRTFP1-4). Further, the present invention provides a cell transformed with the recombinant vector. *Escherichia coli* transformed 5 with pYIL-KRT1-4 was deposited at KCTC (Korean Collection for Type Cultures) on November 11, 2003, and assigned accession number KCTC 10549BP.

In addition, with respect to insertion analogues of the translational fusion partner proteins of the present 10 invention, the addition of a certain sequence to the whole sequence or a partial sequence exerting activity of the translational fusion partner genes identified using a genomic library or a cDNA library may not affect, or may stimulate, the secretion of a non-producible protein. The 15 present inventors investigated the effect of insertion analogues of the translational fusion partners on the secretion of a non-producible protein. Two insertion analogues of TFP3 (104 amino acids), TFP3-3 (189 amino acids) and TFP3-4 (222 amino acids), reduced secretion 20 yield. In contrast, TFP3-1 prepared by adding an N-glycosylation site of 26 amino acids to TFP3 increased the secretion of G-CSF about three times compared to TFP3. Also, TFP3-1-1 (134 amino acids), prepared by adding 4 25 amino acids to TFP3-1, and TFP3-1-2 (143 amino acids), prepared by adding 13 amino acids to TFP3-1, greatly reduced levels of a fusion protein not processed by Kex2p

during secretion without a decrease in the secretion yield of G-CSF. Thus, insertion analogues of the translational fusion partners of the present invention, which have additions of a N-glycosylation site and a region allowing 5 the approach of Kex2p proteinase for cleavage of a fusion site, are included in the scope of the present invention, as long as they do not negatively affect the secretion of a non-producible protein.

In a detailed aspect, the present invention provides 10 a translational fusion partner TFP3-1-1 protein represented by SEQ ID NO. 40 or an analogue thereof. Also, the present invention provides a gene encoding a translational fusion partner TFP3-1-1 protein represented by SEQ ID NO. 40 or an analogue thereof. The scope of the present invention 15 includes a translational fusion partner TFP3-1-1 protein having an amino acid sequence represented by SEQ ID NO. 40 or an amino acid sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. Also, the 20 scope of the present invention includes a gene having a DNA sequence encoding a translational fusion partner TFP3-1-1 protein represented by SEQ ID NO. 40, or a DNA sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or 25 higher homology. Preferably, the gene is a gene of SEQ ID NO. 41. Further, the present invention provides a

recombinant vector comprising the gene. Preferably, the gene carried in the recombinant vector is a gene of SEQ ID NO. 41. An illustrative example of the recombinant vector is pYGT3-1-1-GCSF. Still further, the present invention 5 provides a cell transformed with the recombinant vector. *Escherichia coli* transformed with pYGT3-1-1-GCSF was deposited at KCTC (Korean Collection for Type Cultures) on December 21, 2004, and assigned accession number KCTC 10753BP.

10 In another detailed aspect, the present invention provides a translational fusion partner TFP3-1-2 protein represented by SEQ ID NO. 42 or an analogue thereof. Also, the present invention provides a gene encoding a translational fusion partner TFP3-1-2 protein represented 15 by SEQ ID NO. 42 or an analogue thereof. The scope of the present invention includes a translational fusion partner TFP3-1-2 protein having an amino acid sequence represented by SEQ ID NO. 42 or an amino acid sequence homologous thereto, having preferably 75%, more preferably 85%, even 20 more preferably 90% and most preferably 95% or higher homology. Also, the scope of the present invention includes a gene having a DNA sequence encoding a translational fusion partner TFP3-1-2 protein represented by SEQ ID NO. 42, or a DNA sequence homologous thereto, having preferably 25 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology. Preferably, the gene is

a gene of SEQ ID NO. 43. Further, the present invention provides a recombinant vector comprising the gene. Preferably, the gene carried in the recombinant vector is a gene of SEQ ID NO. 43. An illustrative example of the 5 recombinant vector is pYGT3-1-2-GCSF. Still further, the present invention provides a cell transformed with the recombinant vector. *Escherichia coli* transformed with pYGT3-1-2-GCSF was deposited at KCTC (Korean Collection for Type Cultures) on December 21, 2004, and assigned accession 10 number KCTC 10754BP.

The term "homologous", as used for a translational fusion partner protein or gene herein, is intended to express similarity to the wild-type amino acid sequence and the wild-type nucleotide sequence. In case of the protein, 15 "homologous" includes an amino acid sequence preferably 75% or higher, more preferably 85% or higher, even more preferably 90% or higher and most preferably 95% or higher identical to an amino acid sequence of the TFP protein of the present invention. Typically, protein homologues may 20 include an active site identical to a target protein. In the case of the gene, "homologous" includes a DNA sequence preferably 75% or higher, more preferably 85% or higher, even more preferably 90% or higher and most preferably 95% or higher identical to a DNA sequence encoding the TFP 25 protein of the present invention. The homology evaluation may be done with the naked eye or using a commercially

available program. Using a commercially available computer program, the homology between two or more sequences may be expressed as a percentage (%), and the homology (%) between adjacent sequences may be evaluated.

5 The translational fusion partners identified according to the present invention for the secretory production of non-producible proteins are used in the form of being fused to a gene encoding a non-producible protein and is inserted into a vector for the secretory production
10 of the non-producible protein. The term "vector", as used herein, refers to a DNA construct that contains a DNA sequence operably linked to regulatory sequences capable of controlling the expression of a protein in a suitable host and sequences introduced for facilitating other genetic
15 manipulation or optimizing the expression of the protein. Such regulatory sequences include a promoter for transcription control, an operator selectively added for transcription control, a suitable mRNA ribosome binding site and sequences controlling termination of
20 transcription/translation. Such a vector for insertion of an exogenous gene may be a plasmid, a virus, a cosmid, or the like. The vector includes cloning vectors and expression vectors. The cloning vector is a replicable plasmid into which exogenous DNA is inserted, and delivers
25 exogenous DNA into host cells transformed therewith. The expression vector typically means a carrier into which a

fragment of exogenous DNA, generally a fragment of double-stranded DNA, is inserted. "Exogenous DNA" refers to heterogeneous DNA that does not naturally occur in host cells. The expression vector is able to replicate independently of host chromosomal DNA in host cells so that inserted exogenous DNA may be produced. As generally known in the art, in order to increase the expression level of a transfected gene in a host cell, the gene should be operably linked to transcription and translation regulatory sequences functional in a host cell selected as an expression system.

The term "transformation", as used herein with respect to transformation using a recombinant vector containing a translational fusion partner, means introducing DNA into a suitable host cell so that the DNA is replicable, either as an extrachromosomal element, or by chromosomal integration. Host cells useful for the transformation according to the present invention may be prokaryotic or eukaryotic. In addition, host cells having high transformation efficiency of foreign DNA and having high expression levels of an introduced DNA may be typically used. Examples of host cells include prokaryotic and eukaryotic cells such as *Escherichia* sp., *Pseudomonas* sp., *Bacillus* sp., *Steptomyces* sp., fungi and yeast, insect cells such as *Spodoptera frugiperda* (Sf9), and animal cells such as CHO, COS 1, COS 7, BSC 1, BSC 40 and BMT 10. Also, yeasts including *Candida*, *Debaryomyces*,

Hansenula, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces*, *Yarrowia* and *Saccharomyces* species may be preferably used as host cells for the large-scale production of a non-producible protein according to the present invention.

5 In still another aspect, the present invention relates to a method of recombinantly producing non-producible proteins using the aforementioned TFP proteins.

The method for the recombinant production of a non-producible protein comprises preparing an expression vector 10 into which a coding gene of the non-producible protein, fused to a gene encoding the TFP protein, is inserted, and culturing a transformant transformed with the recombinant expression vector. In detail, the present invention relates to a method of recombinantly producing a non-producible 15 protein using a protein having an amino acid sequence represented by SEQ ID NO. 1, 3, 5, 7, 9, 40 or 42 or an amino acid sequence homologous thereto, having preferably 75%, more preferably 85%, even more preferably 90% and most preferably 95% or higher homology, or using a protein 20 having an amino acid sequence encoded by a gene represented by SEQ ID NO. 10 or an amino acid sequence homologous thereto, having preferably 75% or higher, more preferably 85% or higher, even more preferably 90% or higher and most preferably 95% or higher homology. Preferably, the protein 25 represented by SEQ ID NO. 1 is encoded by a gene represented by SEQ ID NO. 2, the protein represented by SEQ

ID NO. 3 is encoded by a gene represented by SEQ ID NO. 4, the protein represented by SEQ ID NO. 5 is encoded by a gene represented by SEQ ID NO. 6, the protein represented by SEQ ID NO. 7 is encoded by a gene represented by SEQ ID NO. 8, the protein represented by SEQ ID NO. 40 is encoded by a gene represented by SEQ ID NO. 41, and the protein represented by SEQ ID NO. 42 is encoded by a gene represented by SEQ ID NO. 43.

A better understanding of the present invention may 10 be obtained through the following examples which are set forth to illustrate, but are not to be construed as the limit of the present invention.

EXAMPLE 1: Preparation of invertase-deficient yeast mutant

For rapid screening of translational fusion partners 15 of non-producible proteins, an automatic screening system was established through the evaluation of cell growth in a sucrose medium using yeast invertase as a reporter.

A yeast strain deficient for invertase activity was required to use the invertase gene contained in a vector as 20 a reporter gene upon screening after transformation. Thus, the *INV2* gene was deleted in yeast chromosomal DNA. In order to prepare a cassette for inducing gene deletion, a pRB58 plasmid (Carlson et al., Cell, 1982, 20, 145) was digested with *Eco*RI and *Xho*I, and an *INV2* coding gene was

recovered and introduced into *EcoRI/XhoI* sites of pBluescript KS+ (Stratagene, USA), thus generating pBI Δ BX. As shown in FIG. 1, an *URA3* gene having a repeat sequence of 190 bp (Tc190) at both its ends was inserted into 5 *HindIII-XbaI* sites of the *INV2* gene contained in the pBI Δ BX, thus generating pBIU. The pBIU was digested with both *EcoRI* and *XhoI*, and was transformed into *Saccharomyces cerevisiae* Y2805 Δ gall (*Mat a ura3 INV2 pep4::HIS3 gall can1*) strain (SK Rhee, Korea Research Institute of 10 Bioscience and Biotechnology). The transformant, Y2805 Δ gall Δ inv2U (*Mat a inv2::URA3 pep4::HIS3 gall can1*), was selected in a selection medium lacking uracil.

The selected transformed cells were evaluated to determine whether they completely lost invertase activity. 15 Single colonies were cultured in two media containing glucose and sucrose, respectively, as the sole carbon source. As a result, the colonies grew normally in the glucose medium, but grew very slowly in the sucrose medium compared to a control. In order to investigate the amount 20 of invertase secreted into the culture medium, *INV2+* strain and Δ inv2 strain were cultured. Proteins contained in the culture supernatants were separated on SDS-PAGE, and the gel was incubated in a sucrose solution for 30 min and subjected to zymogram analysis using a dye, TTC (2,3,5-triphenyl-tetrazolium chloride). As shown in FIG. 2, the 25 Δ inv2 strain was found to lose most of its invertase

activity. However, the mutant strain had a problem of growing even at very slow rates in the sucrose medium. This is believed to be because cells partially grow by gluconeogenesis through the function of mitochondria. Thus, 5 to solve this problem, antimycin A, an inhibitor of mitochondrial electron transport, was added to the medium to block cell growth. As a result, the growth of the mutant strain was completely inhibited in the presence of antimycin A (FIG. 3).

10 In order to re-transform the selected strain, Y2805 Δ gall1 Δ inv2U (*Mat a inv2::URA3 pep4::HIS3 gall can1*), with a *URA3* vector containing a TFP library, it was necessary to remove the *URA3* gene used for the deletion of the *INV2* gene. To do this, cells were cultured in a medium 15 containing 5-fluoroorotic acid (5-FOA) and selected for loss of the *URA3* gene, thus obtaining a *URA3* pop-out deletion strain, Y2805 Δ gall1 Δ inv2(*Mat a ura3 inv2::Tc190 pep4::HIS3 gall can1*) (FIG. 1). Southern blotting was carried out to confirm the deletion of the *INV2* gene on 20 chromosome, as expected, and the pop-out of the *URA3* gene (FIG. 4). When chromosomal DNA from *S. cerevisiae* Y2805 was treated with *Eco*RI and analyzed by Southern blotting using an *INV2* gene as a probe, a fragment of about 4.3 kb was detected. This size increased to about 5.0 kb when a *URA3* 25 gene was inserted (Y2805 Δ gall1 Δ inv2U), and decreased to about 3.7 kb when the *URA3* gene was popped-out

(Y2805 Δ gal1 Δ inv2). As shown in FIG. 4, as expected, the INV2 gene was obviously deleted, and the URA3 gene was lost (pop-out).

EXAMPLE 2: Identification of automatic screening system
5 through fusion with invertase

The invertase gene-deficient strain was evaluated for the possibility of being automatically screened in a sucrose medium through the expression of a protein fused to invertase, using a human protein expressed in high levels 10 in yeast, human serum albumin (HSA), and a non-producible protein, human interleukin-2 (IL-2).

First, a pGHSA-INV2 vector in which albumin is fused to invertase was prepared as follows. In order to insert a SfiI recognition sequence into both ends of the HSA gene, 15 PCR was carried out using a sense primer and an anti-sense primer, each of which has a SfiI recognition sequence, JH97 (Sfi-HSA-forward primer) (SEQ ID NO. 11) and JH119 (Sfi-HSA-reverse primer) (SEQ ID NO. 12), respectively, pYHSA5 (Kang et al., J. Microbiol. Biotechnol., 1998, 8, 42) as a 20 template, and Pfu polymerase (Stratagene, USA). PCR conditions included one cycle of 94°C for 5 min, and 25 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 2 min, followed by one final cycle of 72°C for 7 min. A PCR product of about 1.8 kb, which was an albumin gene, was

obtained. Separately, the invertase gene was amplified by PCR using a set of primers, JH99 (*Sfi*-INV-forward) (SEQ ID NO. 13) and JH100 (*Sal*I-INV-reverse primer) (SEQ ID NO. 14), and pRB58 as a template under the same conditions. The 5 amplified invertase gene was treated with *Sfi*I/*Sal*I, and was inserted along with the albumin gene treated with *Pst*I/*Sfi*I into *Pst*I/*Sal*I-digested pBluescript (Stratagene, USA). Then, pYHSA5 was digested with *Sac*I/*Pst*I to excise a fragment containing a *GAL* promoter and a portion of the 10 albumin gene. This fragment, and a *Pst*I-*Sal*I insert excised from the plasmid prepared above, containing a portion of the albumin gene and the invertase gene, were co-ligated into the *Sac*I/*Sal*I sites of a YEG α -HIR525 vector (Choi et al., Appl Microbiol Biotechnol., 1994, 42, 587), thereby 15 generating pGHSAs-INV2.

A fusion expression vector of IL-2 and invertase was prepared as follows. The IL-2 gene was amplified by PCR using a set of primers, JH106 (*Sfi*-IL2-forward primer) (SEQ ID NO. 15) and JH107 (*Sfi*-IL2-reverse primer) (SEQ ID NO. 16), and pT7-hIL-2 (JK Jung, Korea Research Institute of 20 Bioscience and Biotechnology) as a template. The amplified interleukin gene was inserted into the *Eco*RV site of pBluescript (Stratagene, USA), thus generating pBKS-IL2. The linearized form of the pBKS-IL2 through *Sfi*I digestion 25 and a *Sac*I-*Sfi*I insert excised from the pGHSAs-INV2, containing a *GAL* promoter and an *INV* secretory signal, were

co-ligated into the *SacI/SfiI* sites of the pGHSAs-INV2, thus generating pGIL2-INV2.

The pGHSAs-INV2 vector expressing a fusion protein of albumin and invertase, the pGIL2-INV2 expressing a fusion protein of IL-2 and invertase, and the pRB58 expressing only invertase were individually transformed into a yeast strain (Y2805 Δ inv2), which is deleted for its endogenous invertase gene and thus unable to grow in a sucrose medium. The transformed cells were smeared onto a medium (UD) containing glucose as a carbon source and a medium (YPSA) containing sucrose as a carbon source, and their growth was observed (FIG. 5). When cells were transformed with the pRB58 vector normally expressing invertase, they normally grew in both carbon sources. Also, when cells were transformed with the pGHSAs-INV2 vector in which invertase is fused to albumin leading to the high-level expression of the invertase, they grew well using both carbon sources. In contrast, when cells were transformed with the pGIL2-INV2 vector in which invertase is fused to IL-2 leading to the poor expression of the invertase, they grew normally on the glucose medium but rarely grew on the sucrose medium. This inability of the pGIL2-INV2-transformed cells to grow in the sucrose medium was believed to result from IL-2 being unable to be secreted from the cells and leads to block the secretion of invertase fused thereto. These results indicate that the use of an exogenous invertase gene,

introduced into a yeast mutant (Y2805 Δ inv2), which cannot grow on a sucrose medium due to deletion of its endogenous invertase gene, and secreted or not therein makes automatic screening of yeast cells possible.

5 EXAMPLE 3: Preparation of translational fusion partner screening vector using a non-producible protein human IL-2

In order to obtain suitable translational fusion partners capable of inducing secretion of a fusion protein using the pGIL2-INV2 vector in which IL-2 is fused to 10 invertase, vectors having three reading frames for preparing a library, pYHTS-F0, F1 and F2, were prepared (FIG. 6).

PCR was carried out using sense primers having three reading frames and a *Bam*HI recognition site, JH120 (*Bam*HI-15 IL2-1-forward primer) (SEQ ID NO. 17), JH121 (*Bam*HI-IL2-2-forward primer) (SEQ ID NO. 18) and JH122 (*Bam*HI-IL2-3-forward primer) (SEQ ID NO. 19), an antisense primer, JH123 (INV-1-reverse primer) (SEQ ID NO. 20), pGIL2-INV2 as a template, and Pfu polymerase (Stratagene, USA). PCR 20 conditions included one cycle of 94°C for 3 min, and 25 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 1 min, followed by one final cycle of 72°C for 7 min. PCR products of about 1.2 kb, containing the IL-2 gene and a portion of the invertase gene, were obtained. Separately,

PCR was carried out using a set of primers, JH124 (INV-forward primer) (SEQ ID NO. 21) and JH95 (INV-2-reverse primer) (SEQ ID NO. 22), and pGIL2-INV2 as a template under the same conditions, thus obtaining a fragment of about 0.9 kb containing a portion of the invertase gene. The PCR products were purified from agarose gels. After each of the three 1.2 kb fragments having three reading frames and the 0.9 kb fragment were mixed, secondary PCR was carried out using sense primers, JH120 (SEQ ID NO. 17), JH121 (SEQ ID NO. 18) 및 JH122 (SEQ ID NO. 19), and an antisense primer, JH95 (SEQ ID NO. 22). Three fragments of about 2.1 kb were obtained by agarose electrophoresis. The three recovered 2.1 kb fragments were digested with both *Bam*HI and *Sal*II and individually inserted into pGIL2-INV2 digested with both *Bam*HI and *Sal*II, thus generating pYHTS-F0, F1 and F2.

EXAMPLE 4: Preparation of suitable translational fusion partner library from yeast genome

A translational fusion partner library was prepared using chromosomal DNA from yeast *Saccharomyces cerevisiae* Y2805 (SK Rhee, Korea Research Institute of Bioscience and Biotechnology) and yeast *Hansenula polymorpha* DL-1 (ATCC26012). After each chromosomal DNA was partially digested with *Sau*3AI, DNA fragments ranging from 0.5 kb to 1.0 kb were purified from agarose gels, and ligated with a

mixture of PYHTS-F0, F1 and F2 vectors digested with *Bam*HI and treated with calf intestine phosphatase (FIG. 6). Then, *E. coli* DH5 α was transformed with the ligated DNA, smeared onto ampicillin-containing LB medium (1% Bacto-tryptone, 5 0.5% yeast extract, 1% NaCl), and incubated at 37° for one day. Using the library DNA prepared from the yeast chromosomal DNA, a library of about 5 \times 10⁴ transformants was obtained. All transformants were recovered using sterile distilled water, and library DNA was isolated from the 10 recovered transformants using a plasmid extraction kit (Bioneer, Korea).

EXAMPLE 5: Automatic screening of translational fusion partners suitable for a non-producible protein human IL-2

The library DNA prepared in Example 4 was transformed 15 into yeast *Saccharomyces cerevisiae* Y2805 Δ gall Δ inv2 (*Mat a ura3 inv2::Tc190 pep4::HIS3 gall can1*) using a lithium acetate procedure (Hills et al., Nucleic Acids Res. 1991, 19, 5791). Then, the transformed cells were smeared onto UD minimal medium lacking uracil (0.67% yeast nitrogen base 20 without amino acids, mixture of various amino acids of proper concentrations, 2% glucose), and YPGSA medium (1% yeast extract, 2% peptone, 2% sucrose, 0.3% galactose, 1 μ g/ml antimycin A), and were incubated at 30°C for 5 days. The number of colonies that emerged on each of the media is

given in Table 1, below, in which the number of transformants is compared before and after introduction of translational fusion partners. When yeast cells were transformed with only the vectors (pYHTS-F0, F1 and F2) used for library preparation, about 1×10^4 colonies were formed on the glucose medium, but the cells did not grow on the sucrose medium as expected. In contrast, when yeast cells were transformed with the yeast genome library, about 11 transformants grew on the sucrose medium, indicating that invertase is secreted with the aid of the introduced translational fusion partners.

TABLE 1

DNA introduced into Y2805 $\Delta gal1\Delta inv2$ (host cells)	Number of transformants	
	UD minimal medium (glucose)	YPSGA (sucrose)
-	0	0
pYHTS vectors	$\sim 1 \times 10^4$	0
pYHTS+genomic library (<i>S. cerevisiae</i>)	$\sim 1 \times 10^4$	10
pYHTS+genomic library (<i>H. polymorpha</i>)	$\sim 1 \times 10^4$	1

EXAMPLE 6: Analysis of translational fusion partners

Transformants grown on the sucrose medium were cultured in YPD medium (1% yeast extract, 2% peptone, 2% glucose) for 24 hrs. After the cultured cells were harvested, they were lysed to isolate the introduced plasmids. The isolated plasmids were re-transformed into *E.*

coli. Plasmids were isolated again from the transformed *E. coli*, assessed for gene insertion by restriction mapping, and subjected to DNA sequencing analysis. As a result, the plasmids were found to contain four genes having different sequences, which served as translational fusion partners (Table 2: novel translational fusion partners inserted into plasmids isolated from transformants having grow in sucrose medium).

TABLE 2

Plasmids	Translational fusion partners	Yeast genes	Number of fused amino acids (total amino acid number)	Characteristics
pYHTS-TFP1	TFP1	<i>Yar066w</i>	105(203)	PRE, N-gly, Ser-rich, GPI
pYHTS-TFP2	TFP2	<i>Yar026c</i>	117(169)	PRE, N-gly
pYHTS-TFP3	TFP3	<i>Yj1158c</i>	104(227)	PRE-PRO, O-gly, PIR
pYHTS-TFP4	TFP4	Unknown	50(unknown)	PRE

10 6-1. Translational fusion partner 1 (TFP1)

The present inventors found translational fusion partner 1 (TFP1) (SEQ ID NO. 2) to be capable of effectively secreting a fusion protein of IL-2 and invertase into the extracellular environment. The TFP1 gene was identical to a yeast *S. cerevisiae* gene *Yar066w*. The *Yar066w* gene is similar to the α -1,4-glucan-glucosidase (STA1) gene and encodes a protein containing a glycosyl-phosphatidylinositol (GPI) anchor. The *Yar066w* gene, which is of unknown function, has 70.3% and 72.7% sequence similarities to yeast *S. cerevisiae* genes of unknown

function, Yol155c and Yil169c, respectively. In an amino acid sequence encoded by the Yar066w gene, the region fused to IL-2 consisted of 105 amino acid residues among the total number of 203 amino acid residues, and contained a secretory signal of 23 amino acid residues for protein secretion, an N-glycosylation site and a serine alanine-rich sequence.

6-2. Translational fusion partner 2 (TFP2)

The present inventors found translational fusion partner 2 (TFP2) (SEQ ID NO. 4) to be capable of effectively secreting a fusion protein of IL-2 and invertase into the extracellular environment. The TFP2 gene was identical to a yeast *S. cerevisiae* gene Yar026c. The Yar026c gene is of unknown function. In an amino acid sequence encoded by the Yar026c gene, the region fused to IL-2 consisted of 117 amino acid residues among the total number of 169 amino acid residues, and contained a secretory signal of 19 amino acid residues for protein secretion and three N-glycosylation sites.

20 6-3. Translational fusion partner 3 (TFP3)

The present inventors found translational fusion partner 3 (TFP3) (SEQ ID NO. 6) to be capable of effectively secreting a fusion protein of IL-2 and invertase into the extracellular environment. The TFP3 gene

was identical to a yeast *S. cerevisiae* gene Yjl158c (PIR4/CIS3). The Yjl158c gene encodes an O-mannosylated protein covalently linked to the cell wall. The Yjl158c gene is known as a multicopy suppressor for a mutant 5 deficient in the *Cik1* gene participating in cell division. In an amino acid sequence encoded by the Yjl158c gene, the region fused to IL-2 consisted of 104 amino acid residues among the total number of 227 amino acid residues, and contained a pre-secretory signal of 23 amino acid residues 10 and a pro-secretory signal of 41 amino acid residues for protein secretion, a Kex2p cleavage site containing a sequence of Lys-Arg, and a PIR repeat sequence.

6-4. Translational fusion partner 4 (TFP4)

The present inventors found translational fusion 15 partner 4 (TFP4) (SEQ ID NO. 8) to be capable of effectively secreting a fusion protein of IL-2 and invertase into the extracellular environment. The TFP4 gene is derived from *Hansenula polymorpha* and is of unknown function. The region fused to IL-2 consisted of 50 amino 20 acid residues, which contained a protein secretory signal of 18 amino acid residues.

EXAMPLE 7: Analysis of fusion proteins secreted into culture medium

To assess proteins secreted by yeast cells grown in a sucrose medium, yeast cells containing the four translational fusion partners described in Example 6 were cultured in YPDG medium (1% yeast extract, 2% peptone, 2% glucose, 0.3% galactose) for 40 hrs. After cells were removed, total proteins dissolved in the remaining culture supernatant were precipitated with acetone (final concentration: 40%) and analyzed by SDS-PAGE. However, each translational fusion partner did not appear as a single band because it had excessive glycosylation at a state of being fused to invertase. To solve this problem, invertase was removed from each vector (pYHTS-TFP1, 2, 3 and 4), and a translational termination codon was introduced into the IL-2 gene. In brief, to obtain a fragment including an IL-2 gene containing a *GAL* promoter, a TFP and a translational termination codon from each vector, PCR was carried out using a set of primers, JH132 (*SacI-GAL-forward primer*) (SEQ ID NO. 23) and JH137 (*IL2-Term-reverse primer*) (SEQ ID NO. 24). The amplified gene fragments were individually digested with *SacI/SalI* and inserted into a *SacI/SalI-digested* YEG α -HIR525 vector, thus generating pYIL-TFP1, 2, 3 and 4. The four IL-2 expression vectors were individually transformed into yeast cells. The resulting single colonies were cultured according to the same methods as described above, and the culture supernatants were analyzed by SDS-PAGE (FIG. 7). As shown in FIG. 7, strong protein bands

having different sizes were found in the culture supernatants of yeast cells containing the IL-2 expression vectors except for pYIL-TFP2. These bands were confirmed by Western blotting using an anti-IL-2 antibody (FIG. 7), thus 5 indicating that each secretion-inducing fusion protein is present at a state of being fused to IL-2. However, the size of the fusion proteins on SDS-PAGE was different from that predicted from the molecular weights of each translational fusion partner and the IL-2 gene. This 10 difference was considered to be due to glycosylation of the fusion protein. Thus, each fusion protein was digested with Endo-H and analyzed by SDS-PAGE (FIG. 8). On an amino acid sequence, TFP1 was found to have one consensus N-glycosylation sequence (amino acid residues 28-30), and 15 TFP3 contained a consensus O-glycosylation sequence. TFP4 was found to have no glycosylation sequence. As predicted, in the case of the protein expressed by the pYIL-TFP1, a great decrease was found in molecular weight after Endo-H digestion, indicating that the protein expressed by the 20 pYIL-TFP1 is N-glycosylated. In contrast, there was no change in molecular weight of the protein expressed by the pYIL-TFP3 upon Endo-H digestion because the protein is O-glycosylated. Also, there was no change in molecular weight of the protein expressed by the pYIL-TFP4.

25 EXAMPLE 8: Production of authentic proteins by Kex2p

cleavage in cells

In order to produce the TFP-IL-2 fusion proteins, which were expressed by the vectors used in Example 7 and secreted into the medium, in the authentic form identical to native human IL-2, a cleavage site (Leu-Asp-Lys-Arg) recognized by Kex2p protease, which yeast cells themselves produce, was inserted between a TFP and IL-2 so as to automatically remove the TFP from cells. To introduce a Kex2p cleavage site into the pYIL-TFP1, PCR was carried out 5 using the pYIL-TFP1 as a template with each of two sets of primers, JH132 (SEQ ID NO. 23) and HY22 (TFP1-LDKR-reverse primer) (SEQ ID NO. 25), and HY23 (TFP1-LDKR-forward primer) (SEQ ID NO. 26) and JH137 (SEQ ID NO. 24). A secondary PCR was carried out using as templates the 10 amplified products, a fragment containing a *GAL* promoter and TFP1 and another fragment containing the IL-2 gene, which were electrophoresed and recovered from a gel, with a set of primers, JH132 (SEQ ID NO. 23) and JH137 (SEQ ID NO. 24). The secondarily amplified *GAL* promoter-TFP1-IL2 15 fragment was digested with *SacI/SalI* and inserted into a *SacI/SalI*-digested YEG α -HIR525 vector, thus generating pYIL-KRTFP1. Also, to introduce a Kex2p cleavage site into the pYIL-TFP2, according to the same method as described above, PCR was carried out using the pYIL-TFP2 as a 20 template with two sets of primers, JH132 (SEQ ID NO. 23) 25

and HY20 (TFP2-LDKR-reverse primer) (SEQ ID NO. 27), and HY21 (TFP2-LDKR-forward primer) (SEQ ID NO. 28) and JH137 (SEQ ID NO. 24). A secondary PCR was carried out using two amplified gene fragments as templates with a set of primers, JH132 (SEQ ID NO. 23) and JH137 (SEQ ID NO. 24).
5 The secondarily amplified fragment was digested with *SacI/SalI* and inserted into a *SacI/SalI*-digested YEG α -HIR525 vector, thus generating pYIL-KRTFP2. Further, to introduce a Kex2p cleavage site into the pYIL-TFP3,
10 according to the same method as described above, PCR was carried out using the pYIL-TFP3 as a template with two sets of primers, JH132 (SEQ ID NO. 23) and HY17 (TFP3-LDKR-reverse primer) (SEQ ID NO. 38), and HY18 (TFP3-LDKR-forward primer) (SEQ ID NO. 39) and JH137 (SEQ ID NO. 24).
15 A secondary PCR was carried out using two amplified gene fragments as templates with a set of primers, JH132 (SEQ ID NO. 23) and JH137 (SEQ ID NO. 24). The secondarily amplified fragment was digested with *SacI/SalI* and inserted into a *SacI/SalI*-digested YEG α -HIR525 vector, thus
20 generating pYIL-KRTFP3. Yet further, to introduce a Kex2p cleavage site into the pYIL-TFP4, according to the same method as described above, PCR was carried out using the pYIL-TFP4 as a template with two sets of primers, JH132 (SEQ ID NO. 23) and HY24 (TFP4-LDKR-reverse primer) (SEQ ID NO.
25 29), and HY25 (TFP4-LDKR-forward primer) (SEQ ID NO. 30) and JH137 (SEQ ID NO. 24). A secondary PCR was carried

out using two amplified gene fragments as templates with a set of primers, JH132 (SEQ ID NO. 23) and JH137 (SEQ ID NO. 24). The secondarily amplified fragment was digested with *SacI/SalI* and inserted into a *SacI/SalI*-digested YEG α -HIR525 vector, thus generating pYIL-KRTFP4.

Among the four vectors, pYIL-KRTFP1, pYIL-KRTFP3 and pYIL-KRTFP4 were individually introduced into a yeast 2805 Δ gall Δ inv2 strain. Single colonies were picked and cultured in YPDG medium (1% yeast extract, 2% peptone, 2% glucose, 0.3% galactose) for 40 hrs. After cells were removed, the remaining culture supernatants were subjected to SDS-PAGE. As shown in FIG. 9, secreted proteins were found to have the same size as native human IL-2. Among the three TFPs inducing the secretory production of human IL-2, TFP1 was found to be most effective in the secretory production of authentic IL-2.

The pYIL-KRTFP1, pYIL-KRTFP2, pYIL-KRTFP3 and pYIL-KRTFP4 vectors were deposited at an international depository authority, KCTC (Korean Collection for Type Cultures; 52, Oun-dong, Yusong-ku, Taejon, Korea) on November 11, 2003, and assigned accession numbers KCTC 10544BP, 10545BP, 10546BP and 10546BP, respectively.

EXAMPLE 9: Analysis of characteristics of the translational fusion partner 1 (TFP1)

The TFP1, identified as a translational fusion partner most effectively inducing the secretory production of IL-2, was assessed to determine whether any of specific sequences present on the TFP1 sequence, a secretory signal 5 (a), an N-glycosylation site (b), a serine alanine-rich sequence (c), an additional sequence (d) and a 5'-UTR (5'-untranslated region) (e), directly affects the secretion of the non-producible protein IL-2. To do this, as shown in FIG. 10, deletion mutants of the TFP1 gene, in which each 10 specific sequence was deleted, were prepared. First, to remove the additional sequence (d) having no unique property from the TFP1 sequence, PCR was carried out using the pYIL-KRTFP1 as a template with a set of primers, JH143 (XbaI-TFP1-d-reverse primer) (SEQ ID NO. 31) and JH132 (SEQ 15 ID NO. 23). The amplified DNA fragment contained the TFP1-1, which was deleted in the GAL promoter and the (d) sequence of the TFP1 sequence. To remove the additional sequence (d) and the serine alanine-rich sequence (c) from the TFP1 sequence, PCR was carried out using the pYIL-KRTFP1 as a template with a set of primers, JH142 (XbaI-TFP1-c-reverse primer) (SEQ ID NO. 32) and JH132 (SEQ ID NO. 23). The amplified fragment contained the TFP1-2, which was deleted in the GAL promoter, the (c) sequence and the (d) sequence. Also, to remove the (d) sequence, the (c) 20 sequence and the N-glycosylation site (b) from the TFP1 sequence, PCR was carried out using the pYIL-KRTFP1 as a 25

template with a set of primers, JH141 (XbaI-TFP1-b-reverse primer) (SEQ ID NO. 33) and JH132 (SEQ ID NO. 23). The amplified fragment contained the TFP1-3, which was deleted in the *GAL* promoter and the sequences (c), (d) and (b). To 5 introduce a Kex2p cleavage site into the IL-2 gene, PCR was carried out using the pYIL-KRTFP1 as a template with a set of primers, JH140 (SpeI-XbaI-LDKR-forward primer) (SEQ ID NO. 34) and JH137 (SEQ ID NO. 24). The amplified IL-2 fragment was purified, digested with *Spe*I and *Sal*I, and, 10 along with each of the three obtained fragments (TFP1-1, 2 and 3) digested with *Sac*I and *Xba*I, inserted into a YEG α -HIR525 vector predigested with *Sac*I and *Sal*I, thus generating, as shown in FIG. 10, pYIL-KRT1-1, pYIL-KRT1-2 and pYIL-KRT1-3, respectively. To remove the 5'-UTR of the 15 TFP1, PCR was carried out using the pYIL-KRTFP1 as a template with a set of primers, HY38 (TFP1-UTR-forward primer) (SEQ ID NO. 35) and JH137 (SEQ ID NO. 24). The amplified gene was purified, digested with *Bam*HI/*Sal*I, and ligated along with a *Sac*I/*Bam*HI-digested *GAL10* promoter 20 into a *Sac*I/*Sal*I-digested YEG α -HIR525 vector, thus generating pYIL-KRT1-4 (FIG. 10).

The four plasmids, pYIL-KRT1-1, pYIL-KRT1-2, pYIL-KRT1-3 and pYIL-KRT1-4, were transformed into yeast cells. Single colonies were cultured, and culture supernatants 25 were subjected to SDS-PAGE. As shown in FIG. 11, an IL-2 band was found only in a culture supernatant of cells

transformed with the pYIL-KRT1-3 containing all of the secretory sequence, the N-glycosylation site and the serine/alanine-rich sequence. The IL-2 band was not observed in culture supernatants of cells transformed with
5 the pYIL-KRT1-2 deleted for the serine/alanine-rich sequence and cells transformed with the pYIL-KRT1-1 deleted in both the serine/alanine-rich sequence and the N-glycosylation site. These results indicate that three characteristic sequences (the secretory sequence, the N-
10 glycosylation site and the serine/alanine-rich sequence) present in the TFP1 are required for effectively inducing IL-2 secretion. Also, when the TFP1 was deleted in its 5'-UTR, protein expression levels increased more than about three times.

15 The pYIL-KRT1-3 and pYIL-KRT1-4 vectors were deposited at an international depository authority, KCTC (Korean Collection for Type Cultures; 52, Oun-dong, Yusong-ku, Taejon, Korea) on November 11, 2003, and assigned accession numbers KCTC 10548BP and 10549BP, respectively.

20 EXAMPLE 10: Fermentation production of human IL-2 using the translational fusion partner TFP1-4

A recombinant yeast strain transformed with the pYIL-KRT1-4 vector was cultured in a 5-L jar fermentor by fed-batch culture to be evaluated for its ability to induce the

secretory production of IL-2. A seed culture to be inoculated in the fermentor was cultured in a flask using a seed culture medium (6.7% yeast nitrogen base without amino acids, 0.5% casamino acids and 2% glucose). When the 5 culture using a fermentation culture medium (4% yeast extract, 1% peptone, 2% glucose) as an initial fermentation medium reached an OD₆₀₀ of about 15, a fed-batch medium (15% yeast extract, 30% glucose, 30% galactose) was supplied with various amounts according to cell growth 10 rates. After a culture period of about 64 hrs, the culture reached an OD₆₀₀ of about 200. 5 µl of the medium was collected at the given time points and assessed for secreted proteins by SDS-PAGE (FIG. 12). Compared to a standard sample, about 500 mg/L of IL-2 was found to be 15 secreted into the medium. The IL-2 produced as a secretory product in the yeast fermentation was found to have an amino-terminal sequence of Ala-Pro-Thr-Ser-Ser-Ser through amino-terminal sequence analysis, indicating that it is identical to the native IL-2 secreted in the human body.

20 EXAMPLE 11: Comparison of the translational fusion partners TFP1 to 4 for their capacity to secrete human G-CSF

In order to determine whether the four translational fusion partners (TFP1, 2, 3 and 4) obtained using the non-producible protein human IL-2 are effective in the

secretion of other non-producible human proteins, each of the four TFPs was fused to a non-producible protein, human G-CSF, expressed in yeast cells and assessed for its secretion. The human G-CSF gene was obtained as follows.

5 PCR was carried out using a human cDNA library with a set of primers, JH144 (GCSF-forward primer) (SEQ ID NO. 36) and JH145 (GCSF-reverse primer) (SEQ ID NO. 37). The amplified gene was digested with *Xba*I/*Sal*I and inserted into the *Xba*I/*Sal*I sites of each of pYIL-KRTFP1, 2, 3 and 4, thus

10 generating pYGCSF-TFP1, 2, 3 and 4.

To express human G-CSF in yeast cells, the pYGCSF-TFP1, 2, 3, and 4 vectors were transformed into yeast cells. Single colonies were isolated and cultured, the culture supernatants were subjected to SDS-PAGE and Western blotting using an anti-G-CSF antibody. The results are given in FIG. 13. G-CSF was produced as a secretory product by each of the TFPs, and TFP1 and TFP3 were found to effectively induce the secretory production of G-CSF. In particular, Western blotting with an anti-G-CSF antibody (Chemicon, USA) demonstrated that TPF3 is most effective in the secretory production of G-CSF. Thus, because each of the four TFPs was demonstrated to exert maximal secretion efficiency according to the type of protein, the four TFPs of the present invention were considered to be very useful

15 as translational fusion partners capable of secreting various non-producible proteins other than IL-2 and G-CSF.

20

25

EXAMPLE 12: Fermentation production of human G-CSF using the translational fusion partner TFP3

A recombinant yeast strain transformed with the pYGCSF-TFP3 vector prepared in Example 11 was cultured in a 5-L jar fermentor by fed-batch culture to be evaluated for its ability to induce the secretory production of human G-CSF. A seed culture to be inoculated in the fermentor was cultured in a flask using a seed culture medium (6.7% yeast nitrogen base without amino acids, 0.5% casamino acids and 10 2% glucose). When the culture using a fermentation culture medium (4% yeast extract, 1% peptone, 2% glucose) as an initial fermentation medium reached an OD₆₀₀ of about 15, a fed-batch medium (15% yeast extract, 30% glucose, 30% galactose) was supplied in various amounts according to 15 cell growth rates. After a culture period of about 64 hrs, the culture reached an OD₆₀₀ of about 200. 5 µl of the medium was collected at the given time points and assessed for secreted proteins by SDS-PAGE (FIG. 14). Compared to a standard sample, about 300 mg/L of human G-CSF was found to 20 be secreted into the medium. The human G-CSF produced as a secretory product in the yeast fermentation was found to have an amino-terminal sequence of Thr-Pro-Leu-Gly-Pro through amino-terminal sequence analysis, indicating that it is identical to the native G-CSF secreted in the human

body.

EXAMPLE 13: Analysis of secretion efficiency of derivatives of the translational fusion partner TFP3 for human G-CSF

Since TFP3 was found to be most effective for the optimal secretion of G-CSF in Example 11, the whole TFP3-derived gene, Yjl158c(*CIS3*), was obtained from the yeast *Saccharomyces cerevisiae* genome by PCR. As shown in FIG. 15, six TFP3 derivatives having different length, TFP3-1, 2, 3 and 4, TFP3-1-1 (SEQ ID NO. 40) and TFP3-1-2 (SEQ ID NO. 42), were prepared. The primarily obtained TFP3 consisted of 104 amino acids among a total of 227 amino acids of Yjl158c(*CIS3*). Products having gradually increased length, TFP3-1 (130 amino acids), TFP3-2 (157 amino acids), TFP3-3 (189 amino acids) and TFP3-4 (222 amino acids), were prepared. The TFP3 derivatives were individually linked to the G-CSF gene, and a Kex2p cleavage site was inserted into the fusion site. As shown in the A panel of FIG. 15, the TFP3-1, which additionally contained 26 amino acids compared to TFP3, increased the secretion of G-CSF about three fold because it contained an *N*-glycosylation site compared to TFP3. In contrast, when the length of TFP3 increased further, the secretion rates of G-CSF did not change, or a decrease in the secretion rates of G-CSF was found in the case of TFP3-3 and TFP3-4.

In addition, as shown in the A panel of FIG. 15, the unprocessed form of a TFP3-1-GCSF fusion protein, which was not completely cleaved by Kex2p, was secreted into the medium in a manner increasing with increasing expression 5 and secretion efficiency. This was considered because sugar moieties added to a plurality of O-glycosylation sites present in the fusion site of the two genes interrupted the approach of Kex2p for cleavage of the fusion site. In this regard, TFP3-1-1 (134 amino acids), having an addition of 4 10 amino acids compared to TFP3, and TFP3-1-2 (143 amino acids), having an addition of 13 amino acids compared to TFP3, were prepared and assessed for their capacity to secrete G-CSF and the decrease in expression as a fusion form. As shown in the B panel of FIG. 15, fusion forms of 15 TFP3-1-1 and TFP3-1-2 with G-CSF greatly decreased with no decrease in secretion of G-CSF. These results indicate that secretion rates of a target protein can be more enhanced by the elaborate manipulation of a fusion site between the target protein and each of the obtained TFPs.

20 Two G-CSF expression vectors, pYGT3-1-1-GCSF and pYGT3-1-2-GCSF containing the translational fusion partners TFP3-1-1 and TFP3-1-2, respectively, were deposited at an international depository authority, KCTC (Korean Collection 25 for Type Cultures; 52, Oun-dong, Yusong-ku, Taejon, Korea) on December 21, 2004, and assigned accession numbers KCTC 10753BP and KCTC 10754BP, respectively.

EXAMPLE 14: Secretory production of an industrial enzyme lipase using the translational fusion partner TFP3

A CalB gene obtained by *Xba*I/*Sal*I digestion of pYGA-CalB14 (ES Choi, Korea Research Institute of Bioscience and Biotechnology) was inserted into the *Xba*I/*Sal*I sites of the G-CSF expression vector, pYGCSF-KRTFP3, thus generating pYGT3-CalB14. The pYGT3-CalB14 vector was compared with the pYGA-CalB14 vector for secretion rates of CalB14 according to culture temperature. When CalB14 was produced as a secretory product using TFP3, it was secreted a rate of more than two times higher than the conventional expression system at an optimal culture temperature of 30°C (Table 3: CalB activity according to culture temperature of yeast cells containing each expression vector)

15

TABLE 3

	Lipase activity	
	30°C	22°C
pYGA-CalB14 (AMY)	295	885
pYGT3-CalB14 (TFP3)	1922	874

A recombinant yeast strain containing the pYGT3-CalB14 vector was cultured in a 5-L jar fermentor at an optimal temperature of 30°C by fed-batch culture to be evaluated for its ability to induce the secretory

production of CalB14. When the culture using a fermentation culture medium (4% yeast extract, 1% peptone, 2% glucose) reached an OD600 of about 15, a fed-batch medium (15% yeast extract, 30% glucose, 30% galactose) was supplied with 5 various amounts according to cell growth rates. The recombinant yeast strain very rapidly grew and secreted CalB14 into the medium with high efficiency without a change in culture temperature. Analysis of protein activity and concentration revealed that about 1.5-2.0 g/L of CalB14 10 is secreted into the medium, thereby making the cost-effective large-scale production of CalB14 possible (FIG. 16).

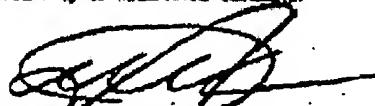
EXAMPLE 15: Evaluation of the activity of the translational fusion partner TFP1 in *Pichia pastoris*

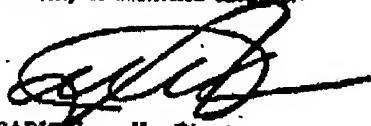
15 In order to determine whether the TFPs developed in the present invention are functional in another yeast, *P. pastoris*, another yeast *P. pastoris* vector, pPIC9 (Invitrogen, USA), was digested with *Bgl*II/*Eco*RI to eliminate the *AOX* promoter, where a *GAP* (glyceraldehyde 3-phosphate dehydrogenase) promoter, obtained using a *Bgl*II-GAP-forward primer (SEQ ID NO. 44) and a GAP-EcoRI-reverse primer (SEQ ID NO. 45), was inserted, thus generating pPIC9-GAP. This vector was digested with *Eco*RI-*Not*I and 20 ligated with each of *Eco*RI-*Not*I-digested Mfalpha (mating

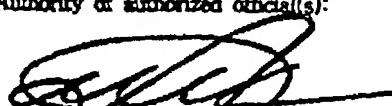
factor alpha)-GCSF and TFP1-GCSF genes, thus yielding pGAP-MF-GCSF and pGAP-TFP1-GCSF. These vectors were individually digested with *Sal*I and transformed into *P. pastoris* GS115 (Invitrogen, USA). The transformed cells were cultured in flasks, and culture supernatants were analyzed by SDS-PAGE for G-CSF secretion to select final transformants. The selected transformants transformed with each of the vectors were batch-cultured in a fermentor using a fermentation medium (4% yeast extract, 1% bactopeptone, 4% glycerol). Samples were collected at given time points, and secretion of G-CSF was analyzed by SDS-PAGE (FIG. 17). As shown in FIG. 17, TFP1 had higher secretion efficiency than Mfalpha. These results indicate that yeast *S. cerevisiae*-derived TFPs are also very useful in *P. pastoris*.

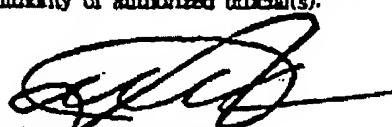
15 Industrial Applicability

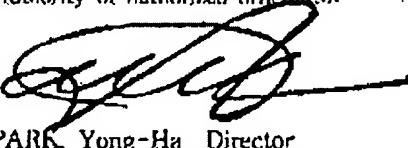
The present invention allows the cost-effective large-scale production of various proteins that are not able to recombinantly produce or are expressed in low levels through the rapid screening and use of suitable TFPs.

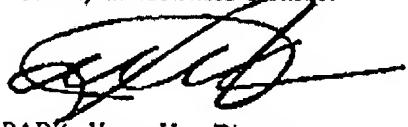
I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> DHE@/pYIL-KRTPP	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: KCTC 10546BP
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
<p>The microorganism identified under I above was accompanied by:</p> <p><input checked="" type="checkbox"/> a scientific description <input type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)</p>	
III. RECEIPT AND ACCEPTANCE	
<p>This International Depository Authority accepts the microorganism identified under I above, which was received by it on November 11 2003.</p>	
IV. RECEIPT OF REQUEST FOR CONVERSION	
<p>The microorganism identified under I above was received by this International Depository Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on</p>	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
<p>Name: Korean Collection for Type Cultures</p> <p>Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) 532, Oum-dong, Yusong-ku, Taejon 305-333, Republic of Korea</p>	<p>Signature(s) of person(s) having the power to represent the International Depository Authority or authorized official(s):</p>  <p>PARK Yong-Ha, Director Date: November 15 2003</p>

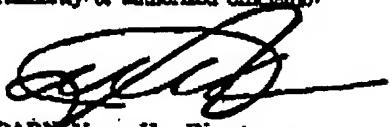
I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> DH5 α /pYIL-KRTFP4	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: KCTC 10547BP
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
<p>The microorganism identified under I. above was accompanied by:</p> <p><input checked="" type="checkbox"/> a scientific description <input type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)</p>	
III. RECEIPT AND ACCEPTANCE	
<p>This International Depository Authority accepts the microorganism identified under I. above, which was received by it on November 11, 2003.</p>	
IV. RECEIPT OF REQUEST FOR CONVERSION	
<p>The microorganism identified under I. above was received by this International Depository Authority on _____ and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on _____</p>	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIIB) #62, Oun-dong, Yusong-gu, Taejon 305-333, Republic of Korea	<p>Signature(s) of person(s) having the power to represent the International Depository Authority or authorized official(s):</p>  <p>PARK Yong-Ha, Director Date: November 15 2003</p>

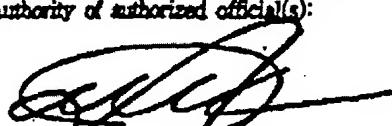
I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> DHS●/pYIL-KRT1-3	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: KCTC 10548BP
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
<p>The microorganism identified under I above was accompanied by:</p> <p>[<input checked="" type="checkbox"/>] a scientific description <input type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)</p>	
III. RECEIPT AND ACCEPTANCE	
<p>This International Depository Authority accepts the microorganism identified under I above, which was received by it on November 11, 2003.</p>	
IV. RECEIPT OF REQUEST FOR CONVERSION	
<p>The microorganism identified under I above was received by this International Depository Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on</p>	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
<p>Name: Korean Collection for Type Cultures</p> <p>Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 306-333, Republic of Korea</p>	<p>Signature(s) of person(s) having the power to represent the International Depository Authority or authorized official(s):</p>  <p>PARK Yong-Ha, Director Date: November 15 2003</p>

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> DE50/pYIL-KET1-4	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: KCTC 10549BP
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
<p>The microorganism identified under I above was accompanied by:</p> <p>[<input checked="" type="checkbox"/>] a scientific description <input type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)</p>	
III. RECEIPT AND ACCEPTANCE	
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IV. RECEIPT OF REQUEST FOR CONVERSION	
<p>The microorganism identified under I above was received by this International Depository Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on</p>	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
<p>Name: Korean Collection for Type Cultures</p> <p>Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oum-dong, Yusong-ku, Taejon 305-333, Republic of Korea</p>	<p>Signature(s) of person(s) having the power to represent the International Depository Authority or authorized official(s):</p>  <p>PARK Jong-Ha, Director Date: November 15 2003</p>

<u>I. IDENTIFICATION OF THE MICROORGANISM</u>	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> DH5 [®] /pYGT3-1-1-GCSF	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: KCTC 10753BP
<u>II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION</u>	
<p>The microorganism identified under I above was accompanied by:</p> <p><input checked="" type="checkbox"/> a scientific description <input type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)</p>	
<u>III. RECEIPT AND ACCEPTANCE</u>	
<p>This International Depository Authority accepts the microorganism identified under I above, which was received by it on Dec 21 2004.</p>	
<u>IV. RECEIPT OF REQUEST FOR CONVERSION</u>	
<p>The microorganism identified under I above was received by this International Depository Authority on _____ and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on _____</p>	
<u>V. INTERNATIONAL DEPOSITORY AUTHORITY</u>	
Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	<p>Signature(s) of person(s) having the power to represent the International Depository Authority or authorized official(s):</p>  <p>PARK, Yong-Ha Director Date: Dec 27 2004</p>

<u>I. IDENTIFICATION OF THE MICROORGANISM</u>	
Identifier reference given by the DEPOSITOR: <i>Escherichia coli</i> UH5@/pYGT3-1-2-GCSF	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: KCTC 10754BP
<u>II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION</u>	
<p>The microorganism identified under I above was accompanied by:</p> <p>[<input checked="" type="checkbox"/>] a scientific description <input type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)</p>	
<u>III. RECEIPT AND ACCEPTANCE</u>	
<p>This International Depository Authority accepts the microorganism identified under I above, which was received by it on Dec 21 2004.</p>	
<u>IV. RECEIPT OF REQUEST FOR CONVERSION</u>	
<p>The microorganism identified under I above was received by this International Depository Authority on and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on</p>	
<u>V. INTERNATIONAL DEPOSITORY AUTHORITY</u>	
Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) #52, Oun-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depository Authority or authorized official(s):  PARK, Yong-Ha Director Date: Dec 27 2004

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> DH5 α /pYIL-KRTFP2	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: KCTC 10645BP
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
<p>The microorganism identified under I above was accompanied by:</p> <p><input checked="" type="checkbox"/> a scientific description <input type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)</p>	
III. RECEIPT AND ACCEPTANCE	
<p>This International Depository Authority accepts the microorganism identified under I above, which was received by it on November 11 2003.</p>	
IV. RECEIPT OF REQUEST FOR CONVERSION	
<p>The microorganism identified under I above was received by this International Depository Authority on _____ and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on _____</p>	
V. INTERNATIONAL DEPOSITORY AUTHORITY	
Name: Korean Collection for Type Cultures Address: Korea Research Institute of Bioscience and Biotechnology (KRIIB) #52, Oon-dong, Yusong-ku, Taejon 305-333, Republic of Korea	Signature(s) of person(s) having the power to represent the International Depository Authority or authorized official(s):  PARK Yong-Ha, Director Date: November 15 2003

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: <i>Escherichia coli</i> DH5α/pYIL-KRTFP1	Accession number given by the INTERNATIONAL DEPOSITORY AUTHORITY: KCTC 10544BP
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
<p>The microorganism identified under I above was accompanied by:</p> <p><input checked="" type="checkbox"/> a scientific description <input type="checkbox"/> a proposed taxonomic designation (Mark with a cross where applicable)</p>	
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V. INTERNATIONAL DEPOSITORY AUTHORITY	
<p>Name: Korean Collection for Type Cultures</p> <p>Address: Korea Research Institute of Bioscience and Biotechnology (KRIBB) 452, Guri-dong, Yusong-ku, Taejon 305-333, Republic of Korea</p>	<p>Signature(s) of person(s) having the power to represent the International Depository Authority or authorized official(s):  PARK Yong-Ha, Director Date: November 15 2003</p>